RESEARCH ARTICLE

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Protective Effect of *Tridax procumbens* Linn Potential in Ulcerative Colitis by Using Myeloperoxidase Activity in Albino Rats

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Abstract: *Tridax procumbens* Linn. (Family: Asteraceae) is being used in traditional medicine for the prevention and treatment of ulcer. In our previous work on same plant material *Tridax procumbens* Linn for evaluation of anti-ulcer activity by using histopathology and ulcer index on mice and rats it is revealed that methanol extract 100 mg/kg was found to be effective in protection of ulcer as compare to petroleum ether extract. Therefore in present research work our aim was to validate protective effect of methanol extract of *Tridax Procumbens* Linn potential in ulcerative colitis in albino rats by using myeloperoxidase activity. In the present study methanol extract 100 mg/kg of *Tridax procumbens* Linn was validated for ulcer protection activity using myeloperoxidase activity in albino rats. The results of present investigation showed that the myeloperoxidase activity of methanol extract 100 mg/kg was 2.74 U/g which was near about 50% lower than Experimental Control which is 4.74 U/g. standard drug prednisolone 5 mg/kg which shows 0.85 U/g. From the myeloperoxidase activity study it can be concluded that the methanolic extract (100 mg/kg) of whole plant of *Tridax procumbens* Linn shows ulcer prevention and protection activity and may be useful for prevention of ulcerative colitis.

INTRODUCTION

Inflammatory bowel disease encompasses two idiopathic, chronic, inflammatory diseases: Crohn's disease and ulcerative colitis. Crohn's disease and ulcerative colitis are disorders of unknown cause involving genetic and immunological influence on the gastrointestinal tract's ability to distinguish foreign from self-antigens. They share many overlapping epidemiological, clinical and therapeutic characteristics. In some patients, it is not possible to distinguish which form of inflammatory bowel disease is present. There are, however, important pathological and clinical differences that distinguish these inflammatory disease processes. Clinically, Crohn's disease tends to present more frequently with abdominal pain and perianal disease, whereas ulcerative colitis is more often characterized by gastrointestinal bleeding. Cobblestoning mucosa and aphthous or linear ulcers characterize the endoscopic appearance of Crohn's disease. Ulcerative colitis presents with diffuse continuous involvement of the mucosa. Radiographic studies of patients with Crohn's disease characteristically show fistulas, asymmetry and ileal involvement. In contrast, radiographic studies of patients with ulcerative colitis show (Figure 1) continuous disease without fistulizing or ileal disease. [1]

Tridax procumbens L. is a common medicinal herb used by ethno-medical practitioners, belonging to family Asteraceae. It is best known as a widespread weed and pest plant. It is native to tropical America but it has been introduced to tropical, subtropical and mid temperate regions worldwide. The plant is a procumbent herb and is valued for its pharmaceutical properties. [2, 3]

Tridax procumbens L. is commonly known as 'Ghamra' and in English popularly known as 'coat buttons' because of the appearance of its flowers. Tridax plant is present throughout India and is employed as indigenous medicine for variety of ailments. It has been extensively used in Ayurvedic system of medicine and is dispensed as "Bhringraj" by some practioners of Ayurveda which is well known medicine for liver disorder. It has been found to possess significant medicinal properties against blood pressure, bronchial catarrh, malaria, dysentery, diarrhea, stomach ache, headache, wound healing, it also prevents hair fall and check hemorrhage from cuts and bruises. [4] Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties. [3, 5] The plant also shows various pharmacological activities like immunomodulatory, antidiabetic, anti hepatotoxic and anti-oxidant, anti-inflammatory, analgesic and marked depressant action on respiration. [5-11]

MATERIALS AND METHODS

Experimental Design

Twenty healthy albino rats of the species *R. norvegicus*, weighing 150–200 g, were used in the study and were divided into four groups with five animals in each group (n = 5) as follows:

1. Group A (normal control) – received 3% gum acacia 10 ml/kg/day p.o.
2. Group B (experimental control) – received 3% gum acacia 10 ml/kg/day p.o.
3. Group C (test) – received methanolic extract of *Tridax procumbens* Linn (METP) 100 mg/ kg/day p.o.
4. Group D (standard) – received prednisolone 5 mg/kg/day p.o.

Preparation of Standard Drug and Extract Solution

Solution of extracts was prepared in 5 % polyethylene glycol. Test drug was dissolved in distilled water or in physiological salt solution. Prednisolone was dissolved in physiological saline.

Plant Material

The plant of *Tridax procumbens* L. (Asteraceae) were identified and located as a tree near Anjneri hills at Trimbakeshwar, District Nashik, MS, India, with the help of botanist and drugs identity was confirmed by comparing

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Table 1: Myeloperoxidase Activity of *Tridax procumbens* on the Colon Tissues

Treatment Group	Myeloperoxidase Activity in Blood U/g
Normal Control	0.48
Experimental Control	4.74
METP 100 mg/kg (Test)	2.45
Prednisolone 5 mg/kg (Std)	0.85

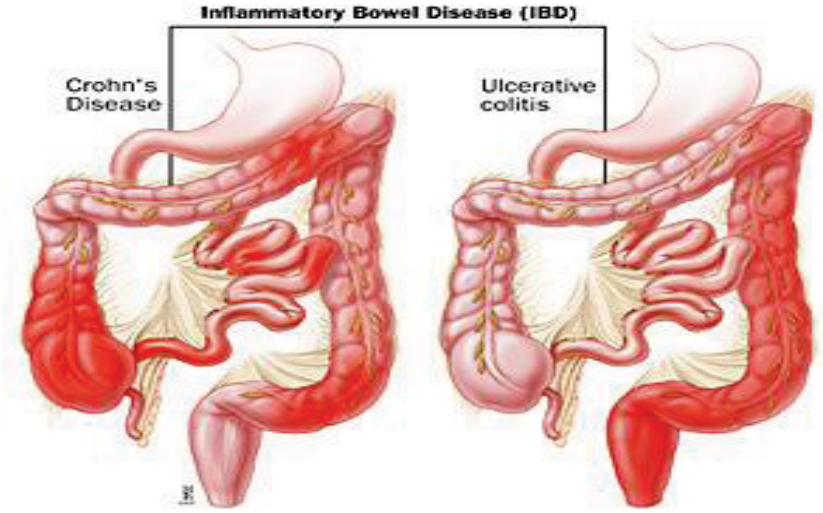

Figure 1: Anatomic distribution of crohn's disease and ulcerative colitis

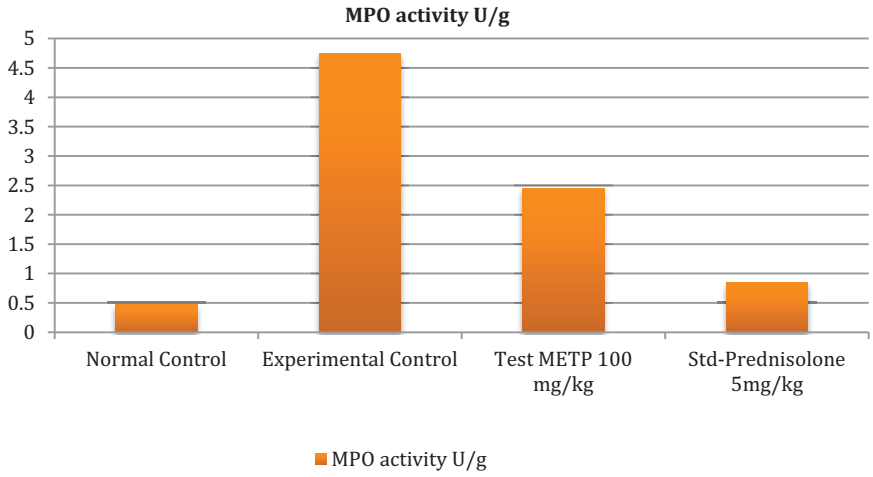

Figure 2: Myeloperoxidase activity of *Tridax procumbens* on the colon tissues

the samples with the description mentioned in the different floras and text. Further to authenticate the plant species herbarium sheet was prepared and forwarded to BSI (Botanical Survey of India, Pune). A voucher specimen (PAR 01) deposited at BSI. Whole plant was collected in the month of September cleaned thoroughly, dried at 30-40°C in oven and coarse powder of it was stored at 25°C in air tight container.

Drugs and Chemicals

Prednisolone were purchased from Medico remedies, Mumbai, India. While methanol, acetic acid, sodium CMC, gum acacia, buffer solution, potassium phosphate buffer myeloperoxidase, hexadecyl trimethyl ammonium bromide, hydrogen peroxide, eosin were purchased from Himedia, India.

Acute Toxicity Study

Acute oral toxicity test for the methanolic extract of whole plant of *Tridax procumbens* Linn was carried out as per Organization for Economic Cooperation and Development (OECD) Guidelines 425. One arbitrary dose of 500 mg/kg was selected for the study, as the extract was found safe even at doses more than 2000 mg/kg without any sign of toxicity or mortality. [12, 13]

Induction of Colitis

The experiment was performed using acetic acid for inducing colitis. All the animals were pre-treated with the respective drugs (volume of drugs was kept constant at 10 ml/kg) for 5 days, along with the normal diet. On the fifth day, animals were fasted for 12 hours (overnight) and IBD was induced the next morning in Groups B, C and D by

administration of 1 ml of 4% acetic acid solution transrectally. Group A (normal control) received 0.9% normal saline transrectally instead. IBD induction was done using an 8-mm soft paediatric catheter which was advanced 6 cm from the anus under low-dose ether anaesthesia. Rats were in trendelenburg position during this process and 1 ml of 4% acetic acid or 0.9% normal saline solution was slowly administered transrectally. The rats were maintained in head-down position for 30 seconds to prevent leakage. After this process, 2 ml of phosphate buffer solution of pH 7 was administered transrectally. [14]

All the animals were sacrificed after 48 hours of IBD induction, by ether overdose. Abdomens were opened and colons were exposed. Distal 10 cm of colon was excised and opened by a longitudinal incision. After washing the mucosa with saline solution, mucosal injury was assessed macroscopically. A 6–8 mm sample block of the inflamed colonic tissue with full thickness was excised from a region of grossly visible damage for histological analysis.

Preparation of the Sample

The proximal 5 cm of the dissected colon specimen was used for biochemical analysis of myeloperoxidase (MPO). The colonic samples were minced and homogenized using a polytron homogenizer. The supernatant was obtained by centrifuging at 3000 rpm for 20 minutes.

Myeloperoxidase Activity

The minced colonic samples were homogenized in 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 6) containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB). The homogenates were then sonicated and centrifuged for 20 minutes at 12,000 rpm. MPO activity was measured spectrophotometrically as follows. Exactly 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer containing 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically at 460 nm. One unit of MPO activity is defined as the change in absorbance per minute at room temperature, in the final reaction. [15]

MPO activity (U/g) = X/weight of the piece of tissue taken, where X = 10×change in absorbance per minute/ volume of supernatant taken in the final reaction. [15]

Statistical Analysis

For all the above methods, the results were expressed as mean±SEM. Statistical analysis was done using one-way analysis of variance (ANOVA), followed by Dunnet's $P<0.05$ was considered significant.

RESULTS

As per the observations in Table 1 and Figure 2, Myeloperoxidase activity of methanol extract 100 mg/kg was 2.74 U/g which was near about 50 % lower than Experimental Control which is 4.74 U/g. standard drug Prednisolone 5 mg/kg which shows 0.85 U/g. Thus the methanol extract 100 mg/kg was found to be significant and may be effective in Ptotection of ulcerative colitis in albino rats.

DISCUSSION

In our previous work on same plant material *Tridax procumbens* Linn for evaluation of anti-ulcer activity by using histopathology and ulcer index on mice and rats it revealed that methanol extract 100 mg/kg was found to be effective in protection of ulcer as compare to petroleum ether extract. Therefore in present research work our aim was to validate protective effect of methanol extract of *Tridax Procumbens* Linn potential in ulcerative colitis in albino rats by using myeloperoxidase activity. As per the observations in Table 1 and Figure 2, myeloperoxidase activity of methanol extract 100 mg/kg was 2.74 U/g which was near about 50 % lower than experimental control which was 4.74 U/g, which was found to be significant and may be effective in Protection of ulcerative colitis in albino rats.

CONCLUSION

In present study our aim was to validate the protective effect of methanol extarct of *Tridax Procumbens* Linn potential in prevention and treatment of ulcerative colitis. from the above myeloperoxidase activity study it can be concluded that the methanolic extract (100 mg/kg) of whole plant of *Tridax procumbens* Linn shows ulcer prevention and protection activity and may be useful for prevention of ulcerative colitis.

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